KU LEUVEN

BIOPROCESS TECHNOLOGY & CONTROL BioTeC

This document contains the **post-print pdf-version** of the referred paper:

"Radio requency inactivation of *Salmonella* Typhimurium and *Listeria monocytogenes* in skimmed and whole milk powder"

by **Maria Tonti, Davy Verheyen, Dmytro Kozak, Torstein Skåra, Jan F.M. Van Impe**

which has been archived on the university repository Lirias (https://lirias.kuleuven.be/) of the Katholieke Universiteit Leuven.

The content is identical to the content of the published paper, but without the final typesetting by the publisher.

When referring to this work, please cite the full bibliographic info:

Tonti, M., Verheyen, D., Kozak, D., Skåra, T., Van Impe, J.F.M. (2024). Radio frequency inactivation of Salmonella Typhimurium and Listeria monocytogenes in skimmed and whole milk powder. International Journal of Food Microbiology, 413, 110556.

The journal and the original published paper can be found at:
https://www.sciencedirect.com/science/article/pii/S0168160523004737

The corresponding author can be contacted for additional info.

Conditions for open access are available at:
http://www.sherpa.ac.uk/romeo/

Postprint version of paper published in International Journal of Food Microbiology 2024, vol. 413, nr. 110556.
The content is identical to the published paper, but without the final typesetting by the publisher.

Radio Frequency inactivation of *Salmonella* Typhimurium and *Listeria monocytogenes* in skimmed and whole milk powder

Maria Tonti[a,b,c], Davy Verheyen[a,b,c], Dmytro Kozak[a,b,c],

Torstein Skåra[d], Jan F.M. Van Impe[a,b,c]

[a]BioTeC+ - Chemical and Biochemical Process Technology and Control

KU Leuven, Gebroeders de Smetstraat 1, 9000 Gent, Belgium

[b]OPTEC, Optimization in Engineering Center-of-Excellence, KU Leuven, Belgium

[c]CPMF^2, Flemish Cluster Predictive Microbiology in Foods - www.cpmf2.be

[d]Nofima, P.O. Box 8034, 4068 Stavanger, Norway

maria.tonti@kuleuven.be, davy.verheyen@kuleuven.be, dmytro.kozak@kuleuven.be,

torstein.skara@nofima.no, jan.vanimpe@kuleuven.be

Declarations of interest: none

Correspondence to:

Prof. J. F. M. Van Impe

Chemical and Biochemical Process Technology and Control (BioTeC+)

Department of Chemical Engineering, KU Leuven

Gebroeders de Smetstraat 1, B-9000 Gent (Belgium)

jan.vanimpe@kuleuven.be
Tel: +32-16-32.14.66

Journal homepage: https://www.sciencedirect.com/journal/international-journal-of-food-microbiology

Original file available at: https://www.sciencedirect.com/science/article/pii/S0168160523004737

ABSTRACT

Milk powder is a convenient, shelf-stable food ingredient used in a variety of food products. However, pathogenic bacteria can be present and survive during prolonged storage, leading to outbreaks of foodborne diseases and product recalls. Radio frequency (RF) heating is a processing technology suitable for bulk treatment of milk powder, aiming at microbial inactivation. This study investigates the RF inactivation of *Salmonella* Typhimurium and *Listeria monocytogenes* in two types of milk powder; skimmed milk and whole milk powder. Specifically, the aims were to (i) examine the influence of the powder's composition on bacterial inactivation, (ii) evaluate the response of bacteria with different Gram properties (Gram positive and Gram negative) and (iii) verify the use of *Enterococcus faecium* as a surrogate for the two microorganisms for the specific RF process. In order to examine exclusively the influence of RF, a non-isothermal temperature profile was used, employing solely different RF energy levels to heat the product to the target temperatures. A log-linear model with a Bigelow-type temperature dependency was fitted to the experimental data. *S.* Typhimurium was less susceptible to RF treatments in comparison to *L. monocytogenes*, demonstrating a higher inactivation rate (k) and higher percentage of sublethal injury. A higher k was also observed for both microorganisms in the whole milk powder, indicating that the increased fat content and decreased levels of lactose and protein in the milk powder had an adverse impact on the microbial survival for both pathogens. The surrogate microorganism *E. faecium* successfully validated the microbial response of the two microorganisms to RF treatments. In general, a low heating rate RF-only process was successful in inactivating the two foodborne pathogens in skimmed and whole milk powder by 4 log (CFU/g)**.**

Keywords: Food microbiology, novel technologies, low moisture food, food safety, predictive modelling

Journal homepage: https://www.sciencedirect.com/journal/international-journal-of-food-microbiology

Original file available at: https://www.sciencedirect.com/science/article/pii/S0168160523004737

Postprint version of paper published in International Journal of Food Microbiology 2024, vol. 413, nr. 110556.
The content is identical to the published paper, but without the final typesetting by the publisher.

1 INTRODUCTION

Milk powder is a versatile food ingredient with several benefits. It is highly nutritious and has a long shelf life which facilitates its storage and distribution (Zhong et al., 2017). Milk powder can be used as reconstituted milk or as an additive in various food products, such as puddings, sauces, and baked goods to enhance texture and flavour (Wei et al., 2021). Due to its low water activity (a_w), it is commonly perceived as "safe". The low a_w creates an adverse environment that inhibits bacterial growth. However, several microorganisms can survive for prolonged periods of time, creating challenges with respect to food safety upon rehydration of the product (Dag et al., 2022).

Over the years, several outbreaks have been traced back to contaminated milk powder (ECDPC and EFSA, 2019; Michael et al., 2014). One of the most prevalent foodborne pathogens implicated in these outbreaks is *Salmonella. Salmonella* is known to survive the spray drying process which is the last critical control point for eliminating microbial contamination (Wei et al., 2021). *Listeria monocytogenes* is another microorganism that can withstand the spray drying process and survive in low moisture foods for an extended period of time, even if it hasn't been linked to any known milk powder outbreaks thus far (Ballom et al., 2020; Ly et al., 2019).

The reduction of microbial contamination in food products is typically addressed through conventional thermal processing technologies (Zhang et al., 2021a). Such technologies have certain limitations regarding the heating rate and uniformity, especially when it comes to the treatment of low moisture food products like milk powder. Radio Frequency (RF) heating is an innovative technology which utilises electromagnetic radiation and generates thermal energy within the product (Altemimi et al., 2019). Numerous studies are available on the RF treatment of various low moisture food products, with a particular interest on milk powder (Dag et al., 2020). The milk powder's composition exerts a big influence on the RF treatments (Dag et al., 2019), but the influence on the bacterial survival in milk powders has not been studied yet. Furthermore, RF treatments have been mainly focused on *Salmonella* spp., despite the fact that *L. monocytogenes*, if present, can survive storage up to a year (Ballom et al., 2020). In most of the RF inactivation studies, a common approach involves the initial use of RF to

Journal homepage: https://www.sciencedirect.com/journal/international-journal-of-food-microbiology

Original file available at: https://www.sciencedirect.com/science/article/pii/S0168160523004737

elevate temperatures to a specific target, followed by isothermal holding using (i) hot air for a set duration at the target temperature (Michael et al., 2014; Wang et al., 2016; Wei et al., 2021) or (ii) placing products in insulation boxes (Ballom et al., 2021). Oven holding involving hot air is the most commonly applied strategy, since small temperature decreases can often occur during product transfer to insulation boxes (Ballom et al., 2021). However, the stage of isothermal holding needed to allow time for internal heat to mitigate non-uniform RF heating (i.e., to ensure bacteria in the cold zones receive adequate treatments) can result in overtreating (i.e., too long time at high temperature) of the hot zones, consequently leading to a decline in quality (Wang et al., 2016; Dag et al., 2022). RF-only treatments consisting of a slow RF heating phase and no conventional oven holding phase could be used to better preserve product quality, according to a Low Temperature Long Time (LTLT) strategy (Dominguez-Hernandez et al., 2018; Liu et al., 2022a). However, this strategy requires more research prior to industrial application, since (i) RF has not been used as a sole technology aiming at bacterial inactivation thus far, and (ii) the potential of RF-only dynamic heating has not been fully explored yet. In contrast to typical RF processes involving an oven heating phase, RF heating is more significant in the overall process, and hence, the interaction between RF, the powder properties (e.g. composition) and the specific pathogenic microorganisms (e.g., influence of the bacterial gram properties), becomes a critical aspect to consider. The effect of these influences has not been studies so far, not for RF processing in general, and especially not in cases involving an RF-only dynamic heating process.

The main objective of this study was to elucidate the influence of milk powder composition and bacterial properties on RF inactivation kinetics during a RF-only dynamic RF decontamination process. The specific subobjectives were to (i) compare the RF inactivation kinetics of two microorganisms with different Gram-types (i.e., *S.* Typhimurium and *L. monocytogenes*), (ii) unravel the effect of the powder's composition on the microbial survival (i.e., using skimmed and whole fat milk powder), and (iii) validate the use of the non-pathogenic microorganism *Enterococcus faecium* as a surrogate for the aforementioned microorganisms, food products and RF process. In order to study the influence of these factors solely during RF exposure, a novel, RF-only (non-isothermal) temperature profile was used, not including the typical conventionally heated temperature holding stage. A log-linear model including a

Bigelow-type temperature dependency was fitted to the acquired experimental data in order to systematically quantify the effect of the aforementioned factors.

2 MATERIALS AND METHODS

2.1 Materials

Skimmed milk powder and whole milk powder were purchased from a SA (Oslo, Norway) in 25-kg bags. The composition (wt. %) of the milk powders as provided by TINE SA is shown in Table 1. After opening each package, the powder was distributed in 1-kg plastic bags which were then vacuumed and sealed. Random sampling was performed in triplicate for each bag, to verify the absence of microbial background flora. The samples were plated on Tryptic Soy Agar (TSA) supplemented with 0.6% yeast extract (Merck, Darmstadt, Germany).

2.2 Microorganisms and microbial inoculum preparation

The two pathogenic bacteria used for this study were *S.* Typhimurium ATCC 14028 (isolated from bovine, septicaemic liver), which was obtained from the Gothenburg Bacterial Collection and *L. monocytogenes* LMG 23356 (isolated from Jalisco Cheese), which was obtained from the bacterial collection of Ghent University. The non-pathogenic microorganism *E. faecium* ATCC 8459 was obtained from the NCIMB collection in UK. Stock cultures of each microorganism were stored at -80°C in 2 ml cryovials (Microbank™, Pro-Lab Diagnostics, Canada).

To prepare the microbial inoculum, one bead of the stock culture was transferred to an Erlenmeyer flask containing 20 mL broth, BHI (Merck, Darmstadt, Germany) for *L. monocytogenes* and TSB (Oxoid, Basingstoke Hampshire, England) for *S.* Typhimurium and *E. faecium*. Afterwards, the Erlenmeyer flasks were incubated at optimum temperature for 24 h, i.e., 30°C for *L. monocytogenes* and 37°C for *S.* Typhimurium and *E. faecium*. A purity plate was created by streaking on non-selective agar media. *L. monocytogenes* was plated on BHI (Merck, Darmstadt, Germany) with 10% Agar (Merck, Darmstadt, Germany) and the purity plate was then incubated at 30°C for 48 h. *S.* Typhimurium and *E. faecium* were plated on TSA (Merck, Darmstadt, Germany) and the plate was incubated at 37°C for 24 h. For

each microorganism, four biological replicates were created by taking one individual colony of the purity plate, placing it in an Erlenmeyer flask containing the relevant broth and incubating at optimum temperature for 24 h. A second 24-h preculture was then created by transferring 20 μL of the first preculture to a new Erlenmeyer flask containing broth and incubating for 24 h at optimum temperature. The viable cell density at the end of the incubation was approximately 10^9 CFU/mL.

2.3 Milk Powder inoculation

10 g of the respective milk powder was weighed into a sterile stomacher bag (GRADE, UK) and inoculated by pipetting 0.1 mL of the second microbial preculture on the powder surface. The powder was then hand-massaged for 5 min, taking short breaks to avoid local over-heating caused by friction. The bag was placed inside a desiccator located in a 37°C incubator for 24 h (adaptation phase). The aim was to provide appropriate time for the bacteria to adapt to the new environment whilst maintaining the original water content of the sample. After the adaptation phase, the a_w values measured by a a_w meter (Aqualab 4TE, METER Group AG, Germany) were 0.256 ± 0.007 and 0.276 ± 0.006 (measured at 25°C) for the inoculated skimmed and whole milk powder respectively, both falling within ± 0.02 of the values of the non-inoculated powder. a_w plays a significant role in the RF heating rate and temperature uniformity (Altemimi et al., 2019), as well as the bacterial thermal resistance (Liu et al., 2018a). Thereafter, the bags were hand-massaged again for one minute to ensure sample inoculation uniformity. By the end of the adaptation phase, the viable cell density of *L. monocytogenes* and *E. faecium* was approximately 10^6 CFU/g and for *S.* Typhimurium it was approximately 10^5 CFU/g. Hence, an extra step was added to increase the viable cell density of *S.* Typhimurium to 10^6 CFU/g, allowing the same initial starting level for all microorganisms. Specifically, 10 mL of the second preculture was centrifuged at 5000×g at 4°C for 10 min. The precipitate was resuspended in 1 mL of TSB and the concentrated bacterial suspension was used to inoculate the milk powder.

The inoculated milk powder was placed in a 35x10 mm container; a sterile, polystyrene petri dish with lid (Sarstedt, Oslo, Norway). To ensure that the containers were filled to their full volume and eliminate the presence of any air headspace, 6 g of inoculated skimmed milk powder and 5 g of inoculated whole milk powder (i.e., different masses were employed to counteract density differences between the two

milk powders) were precisely weighed into the container under sterile conditions for each replicate. The closed container with the inoculated powder was sealed by thermal resistant, adhesive tape to prevent moisture transfer with the environment and leakage of the sample during the RF treatments.

2.4 RF processing method of milk powder

A pilot-scale 4 kW, 27.12 MHz, 50 Ω RF set-up (SAIREM, Lyon, France) was used to perform the RF treatments. All samples were subjected to a RF-only treatment process. Specifically, the process aimed at utilising a dynamic RF-only heating profile to inactivate bacteria. The experimental set-up is shown in Figure 1. A Polytetrafluoroethylene (PTFE) tray with rounded edges and dimensions: 430 mm (L) x 330 mm (W) x 50 mm (H) was used for the RF treatments (Figure 1i). The total sample amount was approximately 2.5 kg of milk powder in each process run. Initially, 1 kg of non-inoculated milk powder was spread uniformly in the bottom of the tray. Then, three sealed containers with inoculated milk powder were placed in fixed positions in a removable insert, in the geometric centre of the PTFE tray (Figure 1ii). This position was selected as preliminary experiments had shown more unstable temperature evolution at the edges of the tray compared to the centre, which showed similar temperature profiles. This phenomenon has also been reported in other studies (Liu et al., 2018b; Tiwari et al., 2011). Two containers enclosed milk powder inoculated with pathogenic microorganisms and the third one enclosed milk powder contaminated with the surrogate microorganism. After the positioning of the containers, 1.5 kg non-inoculated milk powder was added to cover the containers uniformly, and ensuring that the top surface was even over the whole tray area (Figure 1iii). The tray was then placed in the centre of the bottom electrode and the electrode height of the movable top electrode was set at 70 mm, which was approximately 30 mm from the sample surface.

The temperature was logged during the process by mounting an optic fiber sensor (FISO Technologies, Smartec SA, Switzerland) inside the surrogate-inoculated sample (Figure 1ii). For the dynamic heating treatments, 2.5 kg of milk powder were first heated to 55°C at a power of 1 kW (0.4 kW/kg), and then heated further at a slower rate at a power of 0.2 kW (0.08 kW/kg) to the different end temperatures. The specific settings allowed for shorter treatment times in the lower temperature range and the switch temperature of 55°C was selected as it did not affect the inactivation of the pathogenic bacteria. The

surface temperature uniformity was verified by infrared (IR) imaging (Testo 871, Maxsievert, Oslo, Norway) after each treatment and the samples were submerged swiftly in an ice bath to rapidly cool down and prevent further inactivation.

2.5 Sampling and Microbial enumeration

The treated and non-treated samples were placed in a sterile stomacher bag where 0.9% saline solution was added at a 1:10 concentration. The rehydrated samples were stomached for 1 min. To determine viable cell counts, the samples were then plated on non-selective and selective media. For *L. monocytogenes*, BHI Agar and PALCAM (Merck, Darmstadt, Germany) were used as non-selective and selective media, respectively. For *S.* Typhimurium, TSA and Xylose Lysine Deoxycholate Agar (XLD) (Merck, Darmstadt, Germany) were used as a non-selective and selective media, respectively. For *E. faecium*, TSA and Slanetz and Bartley (S&B) (Sigma Aldrich, Norway) were used as a non-selective and selective media, respectively. Following plating, the *L. monocytogenes* samples were incubated at 30°C and the *S.* Typhimurium and *E. faecium* samples were incubated at 37°C for 24 h prior to enumeration.

2.6 Mathematical modelling

RF processing frequently exhibits temperature variations between different process runs (Bedane et al., (2018, 2021); Saka et al., (2021); Ling et al., (2016)). In general, variations between process runs can occur due to differences in the sample's initial temperature, differences in the moisture content of the sample, presence of air gaps, sample inhomogeneity, and sample positioning. In order to eliminate these influencing factors, the RF experimental design established that: (i) the inoculation method ensured similar a_w with the non-inoculated milk powder, (ii) the containers with the contaminated powder were filled to their maximum capacity and each time weighed under sterile conditions to minimise experimental errors, (iii) the containers and the tray itself had a fixed position in the RF cavity, (iv) the surrounding bulk milk powder was weighed. Taking all these measures into account, the authors see two possible reasons because of which variations in temperature profiles still occurred. First of all, initial temperature differences in the sample were observed due to the time necessary to weigh the inoculated samples. However, differences in the heating rate were observed even when the initial temperature

between samples differed by only 0.02 °C. Another fact that might contribute to this variation is the overheating of the triode tube, which is an important part of the RF setup. According to Awuah et al., (2014), "one of the most important factors affecting system reliability is the cooling system of the triode tube itself". The prolonged treatment times using this RF-only approach and multiple experimental repetitions could lead to reduced efficiency of the triode, which subsequently affects the electromagnetic field.

The aforementioned variations in temperature profiles can lead to increased uncertainty in predicting the accurate inactivation rates of microorganisms (Kozak et al., 2021). Therefore, to facilitate accurate data interpretation, the temperature profile of each treated sample as well as the initial and the viable cell density after treatment were independently integrated into the model, avoiding the use of an inaccurate average temperature profile. The model employed was a first order kinetics log-linear model with a Bigelow-type temperature dependency (Stumbo, 1973) (Equations 1, 2).

$$\frac{dN(t)}{dt} = -k\left(T(t)\right) \cdot N\,,\; N(t=0) = N0 \qquad (1)$$

$$k\left(T(t)\right) \; = k\left(T_{ref}\right) \cdot 10^{\frac{T(t)-T_{ref}}{z}} \qquad (2)$$

with $N(t)$ (CFU/g), the viable cell density at time t; k (1/min), the inactivation rate, T_{ref} (°C), the reference temperature set at 78°C, $k(T_{ref})$ (1/min), the inactivation rate at the reference temperature, and z (°C), the temperature sensitivity parameter. The reference temperature T_{ref} of 78 °C was selected as such since preliminary modelling trials demonstrated that the selection of a reference temperature did not affect parameter estimations, as long as the reference temperature was selected within the 50-95 °C temperature range. Therefore, the T_{ref} was set to 78°C in order to provide a temperature which lied in the range of the applicable treatments (Verheyen et al., 2019; Akkermans et al., 2020).

The lsqnonlin function of the Optimization Toolbox of MatLab version R2021b (TheMathworks Inc., Natick, USA) was used to calculate the model parameters, minimizing the sum of squared errors. The differential equations of the inactivation model were solved by combining the primary and secondary

model, using the MatLab solver ode45. The Jacobian matrix was used to estimate the standard errors of the model parameters.

2.7 Sublethal Injury

The percentage of the sublethally injured cells (SICs), i.e., a category of cells which have been injured but maintain the ability to grow under favourable conditions was calculated by Equation 3:

$$SI\ (\%) = \frac{N_{non-selective} - N_{selective}}{N_{non-selective}} \cdot 100 \tag{3}$$

where $N_{non\text{-}selective}$ is the viable cell density (CFU/g) measured on non-selective media and $N_{selective}$ the viable cell density (CFU/g) measured on selective media (Verheyen et al., 2019)

2.8 Statistical analysis

Significant differences between experimental results were identified by conducting analysis of variance (ANOVA, single factor) with a 95% confidence level (a = 0.05). The Fisher's Least Significant Difference test was used to determine the means that distinguished amongst one another. The studies were carried out with MatLab R2021a (The Mathworks Inc., Natick, USA). When $P \leq 0.05$, test statistics were deemed significant.

3 RESULTS AND DISCUSSION

The subobjectives of this study were evaluating the effect of Gram properties and powder composition on the RF inactivation of two pathogens, as well as the suitability of *E. faecium* as a surrogate for these pathogens. To achieve these objectives, both lethal (*k*) and sublethal injury (SI) of all microorganisms present in two milk powders was assessed.

The RF inactivation of *S.* Typhimurium and *L. monocytogenes* in the two types of milk powder is illustrated in Figure 2. Overall, a targeted 4 log (CFU/g) reduction was achieved for both microorganisms for the most severe treatment (95°C). While the standard recommended target is a 5 log (CFU/g) reduction, the detection limit of the microorganisms prevented the identification of a higher

level of reduction. The data points acquired by the applied non-isothermal RF treatment indicated absence of residual population and therefore, a log-linear model with a Bigelow-type temperature dependency was fitted to the data. The model parameters are presented in Table 2. The temperature dependency of the inactivation rate *k* is displayed in Figure 3. It is important to clarify that all temperature profiles were considered separately for the model fit, along with the respective initial and final viable cell density. Therefore, the model parameters were a result of all individual temperature profiles used for the model fit, as well as the corresponding initial and final viable cell densities. The relatively high standard deviations originated from the variability of the different temperature profiles between different RF process runs (as explained in 2.6). In order to illustrate the model fit on the figure, however, only the average, minimum and maximum temperature profiles were utilised. Figure 4 depicts the model fit by assuming these temperature profiles. It should be noted that the employed modelling approach allows for all general findings in the current manuscript to be valid over an entire temperature profile range, ensuring a wide applicability. In future studies, when designing RF processes for any specific setup-product combination, research should, however, focus on obtaining repeatable temperature profiles to guarantee food safety when applying the process in the industry. Furthermore, future research should focus on the optimisation of existing RF equipment to better accommodate the specific characteristics of the product being treated.

The SI after RF treatment to target temperatures in skimmed and whole milk powder for the two pathogens is presented in Figure 5. SI is typically defined as any damage that does not result in the organism's death and often leads to stress adaptation as a result (Wesche et al., 2009). When bacterial cells are sublethally injured, they can still survive under certain conditions, but they will not be able to grow in selective culture media (Verheyen et al., 2020).

3.1 Influence of microorganism gram staining properties

S. Typhimurium exhibited a lower *k* and consequently, a higher tolerance to the RF treatments in comparison to *Listeria* in both types of milk powder at temperatures above 60°C (see Supplementary Materials for statistical analysis). A similar trend between the two microorganisms has been documented in different isothermal treatment studies, for various low-moisture food (LMF, products where $a_w < 0.85$

at 25°C) products (Liu et al., 2022b). Rachon et al. (2016) also found *Salmonella* to exhibit varying degrees of higher resistance to thermal treatments than *Listeria* in four different LMFs (confectionery formulation, chicken meat powder, pet food and savoury seasoning). Quinn et al. (2021) reported similar thermotolerances between *L. monocytogenes* and *S.* Typhimurium in wheat flour and in powder infant formula when treated at 85°C. Conversely, in liquid dairy products, a different pattern has been observed; most species of *Salmonella* are generally more susceptible to heat than *L. monocytogenes* (Mackey and Bratchell, 1989; Rukke et al., 2011). *Salmonella's* acquired resistance to heat appears to be linked to a_w, which has been demonstrated by Liu et al. (2018a) and Xie et al. (2021). These studies showed that D-values for *S. Typhimurium* increased considerably with decreasing a_w values. Dhowlaghar et al., (2021) also verified that a_w is inversely related to the heat tolerance of both *Salmonella* and *L. monocytogenes,* with Salmonella exhibiting higher heat resistance than *L. monocytogenes* during isothermal treatments of desiccated shredded coconut. In the current study, *L. monocytogenes* showed an increase in z value, contrary to *S.* Typhimurium. Similar to the traditional z-value, i.e., the temperature difference required to achieve a tenfold decrease in the D-value, the parameter z in this model represents the sensitivity of k to temperature changes. In skimmed milk powder, *L. monocytogenes* had a higher z in comparison to *S.* Typhimurium, indicating that *L. monocytogenes* could be more resistant in the higher temperature range. *S.* Typhimurium also exhibited higher SICs levels in comparison to *L. monocytogenes*. Thus far, the generation of SICs after RF treatment has been recorded in various studies with products such as apple cider (Geveke et al., 2009), red pepper powder (Zhang et al., 2020), broccoli powder (Zhao et al., 2017), walnut shells (Zhang et al., 2021b) and edible seeds (Xu et al., 2022). Since SI is predominantly caused by damage in the outer membrane (Zhao et al., 2017), it was crucial to document the differences between bacteria with different cell wall structure i.e., Gram properties, as it is one step closer to establish the inactivation mechanisms

3.2 Influence of milk powder composition

Both the model parameters in Table 2 and Figure 3 indicate that, for both microorganisms, the inactivation in whole milk powder is significantly higher than in skimmed milk powder. Significant differences ($p<0.05$) between skimmed and whole milk powder are evident above 70°C and 68°C, for

S. Typhimurium and *L. monocytogenes*, respectively. A comparison of the composition of the two milk powders in Table 1 reveals that, along with the difference in fat content, skimmed milk powder contains significantly higher levels of lactose and protein. Therefore, the differences in inactivation rates in the current study were discussed based on the differences in fat, protein and lactose content.

It has commonly been observed that higher fat content results in increased microbial survival in both low and high water activity (a_w) products. Various studies have documented the protective effect of fat in different products (Chhabra et al., 1999; Trimble et al., 2020). Verheyen et al. (2020) documented a more complex relationship between the fat content and the heat resistance of *L. monocytogenes* in fish model systems. Specifically, in emulsions, a positive correlation between fat content and higher inactivation rates was observed, while in gelled systems the opposite relationship occurred. Jin et al. (2018) documented the increased thermal resistance of *S. enterica* in high protein matrices in comparison to high fat matrices, but only at temperatures above 79.5°C. Moreover, Rachon et al. (2016) verified that *Salmonella* shows higher resistance in high protein products than in high sugar products. The protective effect of lactose on the microbial inactivation has also been documented (Ramaswamy et al., 2009; Kim et al., 2017). Ahmad et al., (2022) tested the thermal resistance of *Salmonella* in powders and demonstrated higher D-values in skimmed milk powder in comparison to lactose-free milk powder. The protective effects of lactose and protein, seem to be the most important factor for the observed inactivation behaviour, potentially counteracting the expected influence of fat level based on literature findings. Nevertheless, the complexity of the milk powder's structural environment could lead to different effects of the fat content in comparison to the effects observed in liquid systems.

Even though significantly different, the difference in k values for *Listeria* in the two milk powders at temperatures higher than 68°C was considerably lower than the difference observed in *S.* Typhimurium. At temperatures below 68°C, no significant differences were seen between the types of the milk powder (Supplementary materials). It is challenging to make a meaningful comparison between the inactivation results in the current study and previous RF inactivation literature results, since most studies on the RF treatment of food products utilise the RF only up to a certain target temperature (come-up-time, CUT) and continue heating either with the assistance of hot air or an oven. Concerning conventional thermal inactivation which primarily relies on heat transfer through conduction, a similar pattern between

skimmed and whole milk powder was observed during isothermal treatments using a thermal-death-time sandwich, a dry heat system designed by Lau et al. (2020). Specifically, Wei et al. (2020) isothermally treated *Salmonella*-inoculated skimmed and whole milk powder at 80°C and 0.30 a_w, and reported D values of 9.92 min and 8.11 min, respectively. Additionally, the D values after isothermal treatment at 80°C were slightly higher in skimmed milk powder than whole milk powder for both *Salmonella* and *E. faecium* in the study of Wei et al. (2021).

The complexity of the product's composition i.e., the variety in composition regarding fat, lactose and protein contents poses difficulty in isolating and assessing the individual effect of each component on the bacterial thermal resistance. Moreover, the current study was, to the best of our knowledge, the first to study the effect of milk powder composition on microbial inactivation by an RF-only heating process. The results indicate that a combination of higher lactose and protein content in skimmed milk powder leads to increased thermal resistance for both microorganisms in the higher temperature range (above 68°C).

Even though no significant differences were observed between the two milk powders ($p<0.05$), whole milk powder demonstrated an increasing percentage of SI with increasing temperature, whereas high numbers of SI were already noticed from the beginning of the RF treatments in skimmed milk powder. The rationale for this could be the longer treatment times observed for skimmed milk powder in comparison to the whole milk powder. The microorganisms in skimmed milk powder are exposed to longer periods in the higher temperature range, which may provide an opportunity to activate the stress response adaptation mechanisms. The imminent risk of SICs is that they may undergo morphological changes at a cellular level and strengthen their stress adaptation abilities (Wesche et al., 2009). Therefore the investigation of the acquired traits of recovered SICs should be addressed in future studies. So far, only Jiao et al. (2021) have inspected the recovery and potential cross-protection of sublethally injured *Salmonella* cells in red pepper powder after RF treatment and found no significant differences between regular and recovered cells. Nonetheless, it is vital to verify the lack of acquired resistance to RF treatments in a variety of products, microbial strains and RF processes.

3.3 *E. faecium* surrogate validation

Surrogate microorganisms are frequently used for microbial validation studies. Pathogenic strains pose a high risk when introduced to processing plants and draconian safety measures have to be applied upon handling (Ballom et al., 2020). In this study, *E. faecium* was investigated as a surrogate microorganism. The specific *E. faecium* strain has already been successfully used in numerous studies as a surrogate microorganism for *Salmonella* species and *L. monocytogenes* in LMF in both RF and traditional thermal inactivation studies (Dag et al., 2022; Quinn et al., 2021; Liu et al., 2018b; Villa-Rojas et al., 2017). However, the surrogate microorganism should be validated for various microbial strains and industrial processes (Dag et al., 2020). It is vital to verify that it can successfully mirror the behaviour of different pathogenic species under different conditions in order to build safe pasteurisation techniques (Rachon et al., 2016).

The behaviour of *E. faecium* during RF treatment in skimmed and whole milk powder is illustrated in Figures 6 and 7. Figure 6 depicts the viable cell densities of *E. faecium*, *S.* Typhimurium and *L. monocytogenes* before and after RF treatments. The temperature profiles to obtain the inactivation data points for *E. faecium* were a combination of the profiles documented in the RF inactivation of both *S.* Typhimurium and *L. monocytogenes* (Supplementary materials). A log-linear model was fitted to the inactivation data of *E. faecium* and the model parameters are presented in Table 3. The SI of *E. faecium* after treatment is presented in Figure 7. Overall, *E. faecium* demonstrated higher tolerance to the specific RF treatments than both pathogens and was therefore found to be a reliable surrogate. The trend of higher inactivation rates observed in the whole milk powder for *S.* Typhimurium and *L. monocytogenes* also applies to *E. faecium*. In general, even though there is a slightly higher SI observed in the whole milk powder in comparison to the skimmed milk powder, statistical significant differences ($p<0.05$) are shown only in the treatment up to 80°C. The surrogate also demonstrated considerable lower levels of SICs in comparison to both microorganisms. However, the increasing levels of SI with higher temperatures and the high standard deviation indicate that the current processing method may induce SI, even in the most thermotolerant microorganisms. Furthermore, the long treatment times of this newly designed RF-only process may pose energy and cost limitations for potential industrial applications. The

proposed treatment strategy should be compared to conventional RF treatments including a non-RF temperature holding phase for similar products in terms of energy consumption, cost, inactivation efficiency and its impact on product quality. Further research is necessary in order to evaluate its efficacy across a variety of thermotolerant microorganisms and strains, and to investigate the potential for cell recovery during storage.

4 CONCLUSIONS

In the current study, the influence of bacterial Gram-type (*L. monocytogenes* vs. *S.* Typhimurium) and milk powder (skimmed vs. whole) composition on the inactivation of pathogenic bacteria during a novel RF-only non-isothermal decontamination process of milk powder was investigated. Concerning the influence of bacterial Gram-type, Gram-negative bacteria were proven to be more resistant to the RF treatment, although this could also be linked to the specific high thermal resistance of *S.* Typhimurium at low a_w. Concerning the influence of milk powder composition, bacteria were more thermally resistant in skimmed milk powder than in whole milk powder, probably due to the protective effects of the higher lactose and protein content in the skimmed milk powder outweighing the (possible) protective effects of higher fat content in the whole milk powder. However, in order to better understand the occurring effects for both the Gram-type and milk powder composition, further studies should be conducted, focusing on identifying the exact RF inactivation mechanisms. *E. faecium* was also demonstrated to be a suitable surrogate microorganism for this type of RF-only process, showcasing its potential use in future studies aiming to develop similar processes for other food products. Considering the potential of applying RF-only dynamic heating treatments, future research should focus on establishing uniform temperature distribution to ensure product safety and quality.

5 ACKNOWLEDGEMENTS

This work was funded by the Norconserv Foundation, by the KU Leuven Research Council through project C24/18/046, by the Research Foundation Flanders (FWO) through project G0B4121N and by the EU H2020 research and innovation program under the Marie Skłodowska-Curie grant agreement no. 956126. Author Davy Verheyen was funded by the Research Foundation Flanders (FWO), grant number 1254421N.

6 REFERENCES

Ahmad, N.H., Marks, B.P., Ryser, E.T., 2022. Effect of Lactose and Milk Protein on Thermal Resistance of *Enterococcus faecium* NRRL B-2354 and *Salmonella* in Dairy Powders. J. Food Prot., 85(12), 1865-1874. https://doi.org/10.4315/JFP-22-111

Akkermans, S., Smet, C., Valdramidis, V., Van Impe, J. (2020). Microbial inactivation models for thermal processes. Food Safety Engineering, 399-420. https://doi.org/10.1007/978-3-030-42660-6_15

Altemimi, A., Aziz, S.N., Al-HiIphy, A.R., Lakhssassi, N., Watson, D.G., Ibrahim, S.A., 2019. Critical review of radio-frequency (RF) heating applications in food processing. Food Qual. Saf. 3(2), 81-91. https://doi.org/10.1093/fqsafe/fyz002

Awuah, G.B., Ramaswamy, H.S., Tang, J. eds., 2014. Radio-Frequency heating in food processing: Principles and applications. CRC Press.

Ballom, K.F., Tsai, H.C., Taylor, M., Tang, J., Zhu, M.J., 2020. Stability of *Listeria monocytogenes* in non-fat dry milk powder during isothermal treatment and storage. Food Microbiol. 87, 103376. https://doi.org/10.1016/j.fm.2019.103376

Bedane, T.F., Altin, O., Erol, B., Marra, F., Erdogdu, F., 2018. Thawing of frozen food products in a staggered through-field electrode radio frequency system: A case study for frozen chicken breast

meat with effects on drip loss and texture. IFSET, 50, 139-147. https://doi.org/10.1016/j.ifset.2018.09.001

Bedane, T.F., Erdogdu, F., Lyng, J.G., Marra, F., 2021. Effects of geometry and orientation of food products on heating uniformity during radio frequency heating. Food Bioprod. Process. 125, 149-160. https://doi.org/10.1016/j.fbp.2020.11.010

Chhabra, A.T., Carter, W.H., Linton, R.H., Cousin, M.A., 1999. A predictive model to determine the effects of pH, milkfat, and temperature on thermal inactivation of *Listeria monocytogenes*. J. Food Protect. 62(10), 1143-1149. https://doi.org/10.4315/0362-028X-62.10.1143

Dag, D., Singh, R.K., Kong, F., 2019. Dielectric properties, effect of geometry, and quality changes of whole, nonfat milk powder and their mixtures associated with radio frequency heating. J. Food Eng. 261, 40-50. https://doi.org/10.1016/j.jfoodeng.2019.04.017

Dag, D., Singh, R.K., Kong, F., 2020. Developments in radio frequency pasteurization of food powders. Food Rev. Int. 38(6), 1197-1214. https://doi.org/10.1080/87559129.2020.1775641

Dag, D., Singh, R.K., Chen, J., Mishra, A., Kong, F., 2022. Radio frequency assisted thermal processing for pasteurization of packaged whole milk powder surrounded by oil. Food Control 135, 108762. https://doi.org/10.1016/j.foodcont.2021.108762

Dhowlaghar, N., Tang, J., Zhu, M. J., 2021. Thermal inactivation of *Salmonella*, *Listeria monocytogenes* and *Enterococcus faecium* NRRL B-2354 in desiccated shredded coconut. LWT, 149, 111851. https://doi.org/10.1016/j.lwt.2021.111851

Dominguez-Hernandez, E., Salaseviciene, A., Ertbjerg, P. 2018. Low-temperature long-time cooking of meat: Eating quality and underlying mechanisms. Meat Science, 143, 104-113. https://doi.org/10.1016/j.meatsci.2018.04.032

European Centre for Disease Prevention and Control (ECDPC), European Food Safety Authority (EFSA), 2019. Multi-country outbreak of *Salmonella* Poona infections linked to consumption of infant formula. EFSA supporting publication 2019:EN-1594. 18 https://doi.org/10.2903/sp.efsa.2019.EN-1594

Geveke, D.J., Gurtler, J., Zhang, H.Q., 2009. Inactivation of *Lactobacillus plantarum* in apple cider, using radio frequency electric fields. J. Food Protect. 72(3), 656-661. https://doi.org/10.4315/0362-028X-72.3.656

Jiao, S., Zhang, H., Liao, M., Hayouka, Z., Jing, P., 2021. Investigation of the potential direct and cross protection effects of sublethal injured *Salmonella* Typhimurium induced by radio frequency heating stress. Food Res. Int. 150, 110789. https://doi.org/10.1016/j.foodres.2021.110789

Jin, Y., Pickens, S.R., Hildebrandt, I.M., Burbick, S.J., Grasso-Kelley, E.M., Keller, S.E., Anderson, N.M., 2018. Thermal inactivation of *Salmonella* Agona in low–water activity foods: predictive models for the combined effect of temperature, water activity, and food component. J. Food Prot. 81(9), 1411-1417. https://doi.org/10.4315/0362-028X.JFP-18-041

Kim, S.S., Jo, Y., Kang, D.H., 2017. Combined inhibitory effect of milk fat and lactose for inactivation of foodborne pathogens by ohmic heating. LWT, 86, 159-165. https://doi.org/10.1016/j.lwt.2017.07.043

Kozak, D.S., Tonti, M., Cuba, P., Espitia, J., Tsepelev, V.S., Verheyen, D., Van Impe, J.F. (2021). Design of a Low-Power Radio Frequency Unit and Its Application for Bacterial Inactivation under Laboratory Conditions. Appl. Sci. 11(23), 11117. https://doi.org/10.3390/app112311117

Ling, B., Hou, L., Li, R., Wang, S., 2016. Storage stability of pistachios as influenced by radio frequency treatments for postharvest disinfestations. IFSET 33, 357-364. https://doi.org/10.1016/j.ifset.2015.10.013

Liu, S., Tang, J., Tadapaneni, R.K., Yang, R., Zhu, M.J., 2018a. Exponentially increased thermal resistance of *Salmonella* spp. and *Enterococcus faecium* at reduced water activity. Appl. Environ. Microb. 84(8), 2742-17. https://doi.org/10.1128/AEM.02742-17

Liu, S., Ozturk, S., Xu, J., Kong, F., Gray, P., Zhu, M.J., Sablani, S.S., Tang, J., 2018b. Microbial validation of radio frequency pasteurization of wheat flour by inoculated pack studies. J. Food Eng. 217, 68-74. https://doi.org/10.1016/j.jfoodeng.2017.08.013

Liu, S., Xiong, H., Qiu, Y., Dai, J., Zhang, Q., Qin, W. 2022a. Radiofrequency-assisted low-temperature long-time (LTLT) pasteurization of onion powder. LWT, 170, 114002. https://doi.org/10.1016/j.lwt.2022.114002

Liu, S., Roopesh, M.S., Tang, J., Wu, Q., Qin, W., 2022b. Recent development in low-moisture foods: Microbial safety and thermal process. Food Res. Int. 155, 111072. https://doi.org/10.1016/j.foodres.2022.111072

Ly, V., Parreira, V.R., Farber, J.M., 2019. Current understanding and perspectives on *Listeria monocytogenes* in low-moisture foods. Curr. Opin. Food Sci. 26, 18-24. https://doi.org/10.1016/j.cofs.2019.02.012

Mackey, B.M., Bratchell, N., 1989. The heat resistance of *Listeria monocytogenes.* Lett. Appl. Microbiol. 9(3), 89-94. https://doi.org/10.1111/j.1472-765X.1989.tb00298.x

Michael, M., Phebus, R.K., Thippareddi, H., Subbiah, J., Birla, S.L., Schmidt, K.A. (2014). Validation of radio-frequency dielectric heating system for destruction of *Cronobacter sakazakii* and *Salmonella* species in nonfat dry milk. J. Dairy Sci. 97(12), 7316-7324. https://doi.org/10.3168/jds.2013-7862

Quinn, A.R., Liao, R.F., Steele, F.M., Jefferies, L.K., Taylor, B.J., 2021. Isothermal inactivation of *Salmonella*, *Listeria monocytogenes*, and *Enterococcus faecium* NRRL B-2354 in peanut butter, powder infant formula, and wheat flour. Food Control, 121, 107582. https://doi.org/10.1016/j.foodcont.2020.107582

Rachon, G., Peñaloza, W., Gibbs, P.A., 2016. Inactivation of *Salmonella*, *Listeria monocytogenes* and *Enterococcus faecium* NRRL B-2354 in a selection of low moisture foods. Int. J. Food Microbiol. 231, 16-25. https://doi.org/10.1016/j.ijfoodmicro.2016.04.022

Ramaswamy, H.S., Jin, H., Zhu, S., 2009. Effects of fat, casein and lactose on high-pressure destruction of *Escherichia coli* K12 (ATCC-29055) in milk. food and bioproducts processing, 87(1), 1-6. https://doi.org/10.1016/j.fbp.2008.02.005

Rukke, E.O., Sørhaug, T., Stepaniak, L., 2011. Heat treatment of milk: Thermization of Milk. In J. W. Fuquay et al. (Eds.), Encyclopaedia of dairy science, 2nd edition (693-698). Elsevier.

Saka, I., Topcam, H., Son, E., Ozkaya, B., Erdogdu, F., 2021. Effect of radio frequency processing on physical, chemical, rheological and bread-baking properties of white and whole wheat flour. LWT, 147, 111563. https://doi.org/10.1016/j.lwt.2021.111563

Stumbo, C.R. 1973. Thermobacteriology in Food Processing, 2nd ed. Academic Press, New York.

Sumner, S.S., Sandros, T.M., Harmon, M.C., Scott, V.N., Bernard, D.T., 1991. Heat resistance of *Salmonella* Typhimurium and *Listeria monocytogenes* in sucrose solutions of various water activities. J. Food Sci. 56(6), 1741-1743. https://doi.org/10.1111/j.1365-2621.1991.tb08684.x

Tiwari, G., Wang, S., Tang, J., Birla, S. L., 2011. Analysis of radio frequency (RF) power distribution in dry food materials. J. of Food Eng. 104(4), 548-556.

Tonti, M., Verheyen, D., Kozak, D., Tsakali, E., Skara, T., Van Impe, J.F.M., 2023. Design and validation of a Radio Frequency-only thermal process for skimmed milk powder. *Submitted*

Trimble, L.M., Frank, J.F., Schaffner, D.W., 2020. Modification of a predictive model to include the influence of fat content on *Salmonella* inactivation in low-water-activity foods. J. Food Protect. 83(5), 801-815. https://doi.org/10.4315/0362-028X.JFP-18-431

Verheyen, D., Baka, M., Akkermans, S., Skåra, T., Van Impe, J.F. (2019). Effect of microstructure and initial cell conditions on thermal inactivation kinetics and sublethal injury of *Listeria monocytogenes* in fish-based food model systems. Food Microbiol. 84, 103267. https://doi.org/10.1016/j.fm.2019.103267

Verheyen, D., Govaert, M., Seow, T.K., Ruvina, J., Mukherjee, V., Baka, M., Skåra, T., Van Impe, J.F., 2020. The complex effect of food matrix fat content on thermal inactivation of *Listeria monocytogenes*: Case study in emulsion and gelled emulsion model systems. Front. Microbiol. 10, 3149. https://doi.org/10.3389/fmicb.2019.03149

Villa-Rojas, R., Zhu, M.J., Marks, B.P., Tang, J., 2017. Radiofrequency inactivation of *Salmonella Enteritidis* PT 30 and *Enterococcus faecium* in wheat flour at different water activities. Biosyst. Eng. 156, 7-16. https://doi.org/10.1016/j.biosystemseng.2017.01.001

Wang, L., Tang, J., Lin, Y., Xie, Y., Wang, S., Gao, Z., Liu, Y., 2016. Comparison of *Cronobacter sakazakii* thermal death kinetics in powdered infant formula milk using hot water and combined radio frequency and hot air treatment. ASABE, 2016 Annual International Meeting. https://doi.org/10.13031/aim.20162459966

Wei, X., Lau, S.K., Chaves, B.D., Danao, M.G.C., Agarwal, S., Subbiah, J., 2020. Effect of water activity on the thermal inactivation kinetics of *Salmonella* in milk powders. J. Dairy Sci. 103(8), 6904-6917. https://doi.org/10.3168/jds.2020-18298

Wei, X., Agarwal, S. and Subbiah, J., 2021. Heating of milk powders at low water activity to 95° C for 15 minutes using hot air-assisted radio frequency processing achieved pasteurization. J. Dairy Sci. 104(9), 9607-9616. https://doi.org/10.3168/jds.2021-20449

Wesche, A.M., Gurtler, J.B., Marks, B.P., Ryser, E.T., 2009. Stress, sublethal injury, resuscitation, and virulence of bacterial foodborne pathogens. J. Food Protect. 72(5), 1121-1138. https://doi.org/10.4315/0362-028X-72.5.1121

Xie, Y., Xu, J., Yang, R., Alshammari, J., Zhu, M.J., Sablani, S., Tang, J., 2021. Moisture content of bacterial cells determines thermal resistance of *Salmonella enterica* serotype Enteritidis PT 30. Appl. Environ. Microb. 87(3), 2194-20. https://doi.org/10.1128/AEM.02194-20

Xu, Y., Li, R., Li, K., Yu, J., Bai, J., Wang, S., 2022. Inactivation of inoculated *Salmonella* and natural microflora on two kinds of edible seeds by radio frequency heating combined with cinnamon oil vapor. LWT *154*, 112603. https://doi.org/10.1016/j.lwt.2021.112603

Zhang, H., Zhao, Y., Gong, C., Jiao, S., 2020. Effect of radio frequency heating stress on sublethal injury of *Salmonella* Typhimurium in red pepper powder. LWT 117, 108700. https://doi.org/10.1016/j.lwt.2019.108700

Zhang, L., Lan, R., Zhang, B., Erdogdu, F., Wang, S., 2021a. A comprehensive review on recent developments of radio frequency treatment for pasteurizing agricultural products. Crit. Rev. Food. Sci. Nutr. 61(3), 380-394. https://doi.org/10.1080/10408398.2020.1733929

Zhang, L., Ma, H., Wang, S., 2021b. Pasteurization mechanism of *S. aureus* ATCC 25923 in walnut shells using radio frequency energy at lab level. LWT 143, 111129. https://doi.org/10.1016/j.lwt.2021.111129

Zhao, Y., Zhao, W., Yang, R., Singh Sidhu, J., Kong, F., 2017. Radio frequency heating to inactivate microorganisms in broccoli powder. Food Qual. Saf. 1(1), 93-100. https://doi.org/10.1093/fqsafe/fyx005

Zhong, Y., Wu, Y., Zheng, Y., Zhu, H., Liu, Z., Jiao, S., 2017. Assessment of radio frequency heating on composition, microstructure, flowability and rehydration characteristics of milk powder. Food Sci. Technol. 37, 544-551. https://doi.org/10.1590/1678-457X.18316

TABLES

Table 1: Indicative composition (wt.%) of milk powders, provided by TINE SA

Composition (%)	Skimmed milk powder	Whole milk powder
Fat	0.8	35
Lactose	47.9	33.9
Protein	36.9	23.8
Ash	7.9	5.3
Water	< 5	< 3

Table 2: Model parameters of the log-linear model with a Bigelow type temperature dependency fitted to experimental data of the RF inactivation of *S.* Typhimurium and *L. monocytogenes* in skimmed and whole milk powder (n = 4). Significant differences (P ≤ 0.05) between the microorganisms inoculated in each product are indicated by means of a different lowercase letter. Significant differences (P ≤ 0.05) between the two powders for each microorganism are indicated by means of a different uppercase letter.

Model Parameters	*S.* Typhimurium		*L. monocytogenes*	
	Skimmed milk powder	Whole milk powder	Skimmed milk powder	Whole milk powder
k(78°C) (1/min)	0.035 ± 0.002^{aA}	0.059 ± 0.002^{aB}	0.061 ± 0.003^{bA}	0.068 ± 0.007^{bB}
z (°C)	45.2 ± 13.2^{bA}	34.4 ± 7.6^{aA}	49.8 ± 14.3^{aB}	31.9 ± 9.2^{aA}

Table 3: Model parameters of the log-linear model with a Bigelow type temperature dependency fitted to experimental data of the RF inactivation of *E. faecium* in skimmed and whole milk powder. Separate ANOVA tests were performed for each microorganism and each type of milk powder for all model parameters (n = 2). Significant differences (P ≤ 0.05) between the two powders for each microorganism are indicated by means of a different uppercase letter, with 'A' bearing the lowest value.

Model Parameters	*E. faecium*	
	Skimmed milk powder	Whole milk powder
k(78°C) (1/min)	0.009 ± 0.004^{A}	0.020 ± 0.009^{B}
z (°C)	15.8 ± 5.5^{B}	12.3 ± 4.0^{A}

FIGURE CAPTIONS

Figure 1: (i) PTFE tray (ii) non-inoculated milk powder at the bottom of the tray and samples in the sealed containers (petri dishes) with the optic fibre sensors in the centre of the tray, (iii) covered by non-inoculated milk powder. The container is placed on the bottom electrode in the centre of the RF chamber (iv).

Figure 2: Cell density (log(CFU/g)) of *S.* Typhimurium in skimmed (i) and whole (ii) milk powder and *L. monocytogenes* in skimmed (iii) and whole (iv) milk powder after RF treatment to different end temperatures (n = 4). Data points of black colour represent initial cell density (log(CFU/g)). Data points of different colours represent the cell density (log(CFU/g)) after RF treatment to the respective end temperature. The temperature profile of each treatment corresponds to the specific data points of similar colour. The dashed line represents the detection limit set at 2 log(CFU/g).

Figure 3: The relationship between the inactivation rate *k* (1/min) and temperature *T* (°C) for the two microorganisms in the two milk powders as described by the Bigelow-type equation. *S.*

Typhimurium is depicted with black colour and *L. monocytogenes* with blue colour while skimmed milk powder (SMP) and whole milk powder (WMP) are depicted with solid and dashed lines respectively.

Figure 4: Log-linear model with a Bigelow-type temperature dependency fitted to the experimental data of *S.* Typhimurium in skimmed (i) and whole (ii) milk powder and *L. monocytogenes* in skimmed (iii) and whole (iv) milk powder after RF treatment to different end temperatures (n = 4). The 'x' symbols represent the actual data points used to fit the model. An assumed temperature profile was used to generate the model fit. The solid line was generated by taking a profile created using the average heating rate of all the different temperature profiles, while the lower and upper limits of the shaded area were generated by profiles created using the minimum and maximum heating rates, respectively. The dashed line represents the detection limit set at 2 log(CFU/g).

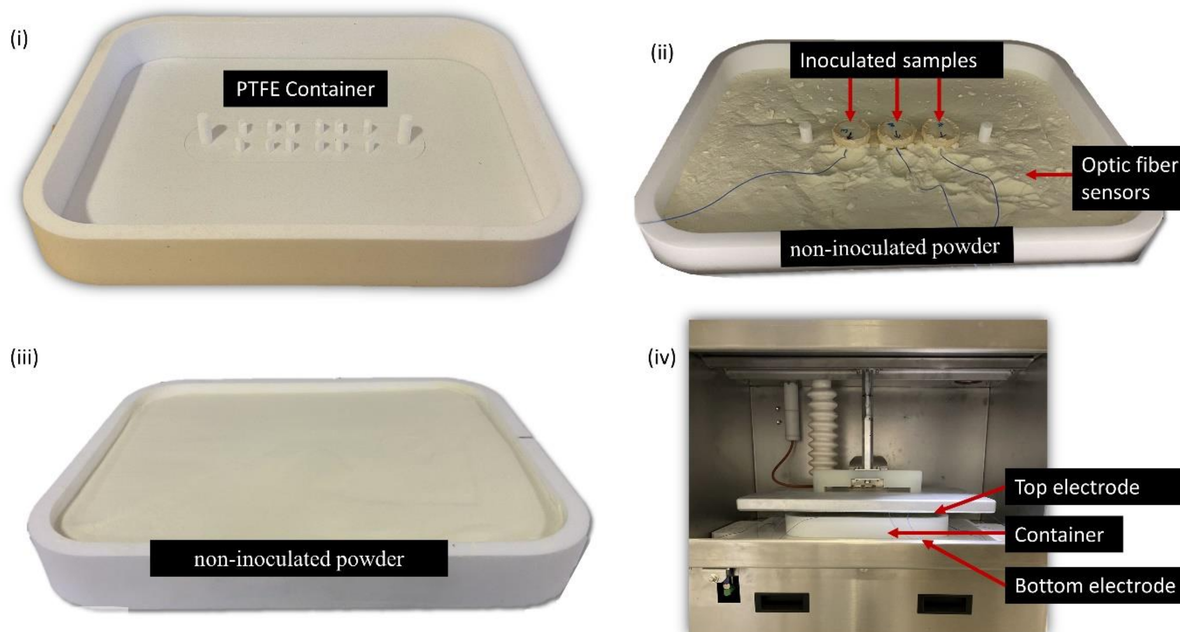
Figure 5: Sublethal Injury (%) of *S.* Typhimurium in skimmed (i) and whole (ii) milk powder and *L. monocytogenes* in skimmed (iii) and whole (iv) milk powder after Radio Frequency treatment. Different lowercase letters represent significant differences ($p<0.05$) between RF treated samples in one type of milk powder and different uppercase letters represent significant differences ($p<0.05$) between the two types of milk powder for each microorganism (n = 4).

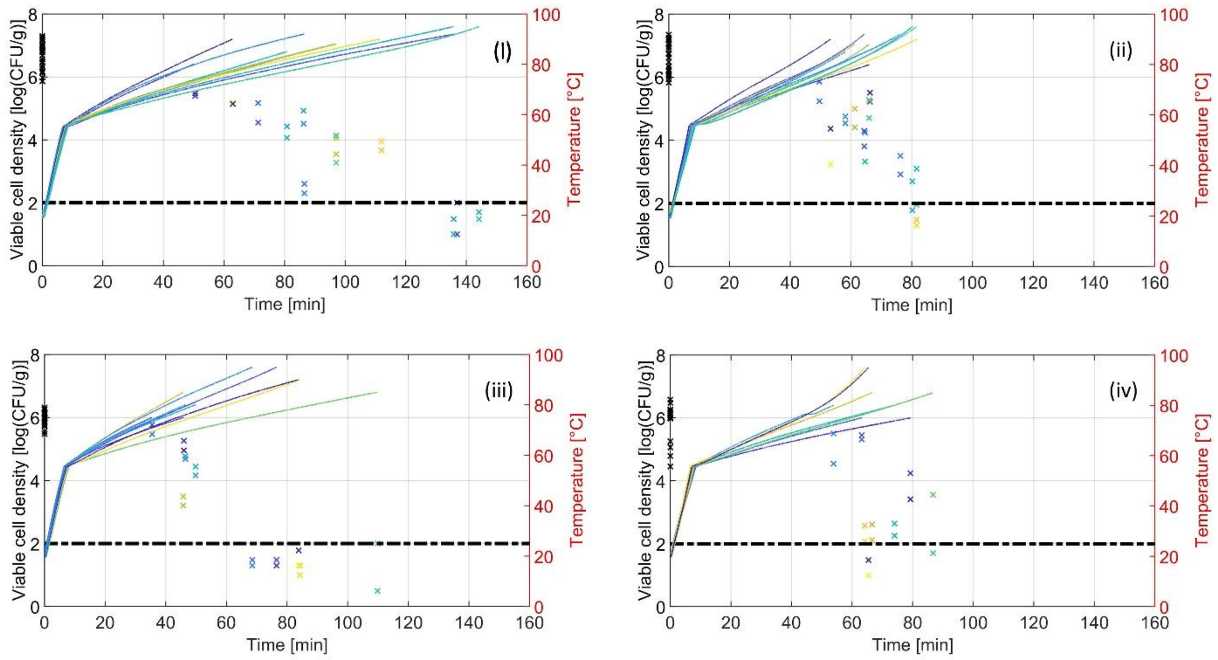
Figure 6: Viable cell density (log(CFU/g)) of *E. faecium*, *S.* Typhimurium, and *L. monocytogenes* in skimmed (i) and whole (ii) milk powder following RF treatment to various end temperatures, depicted by red, blue, and green colors, respectively. The dashed line indicates the detection limit set at 2 log(CFU/g).

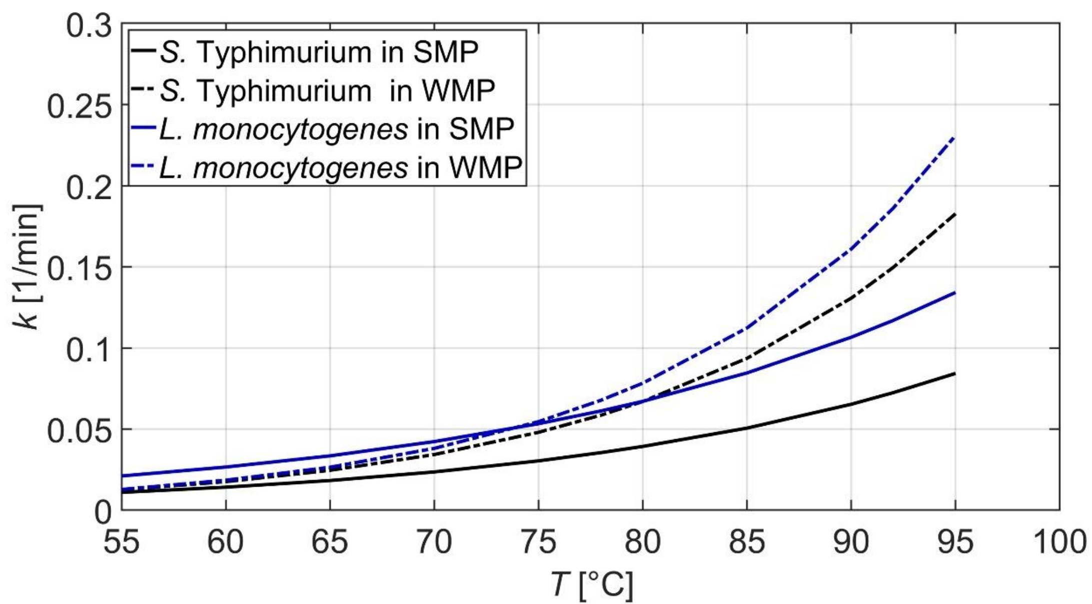
Figure 7: Sublethal Injury (%) of *E. faecium* in skimmed (i) and whole (ii) milk powder after Radio Frequency treatment. Different lowercase letters represent significant differences

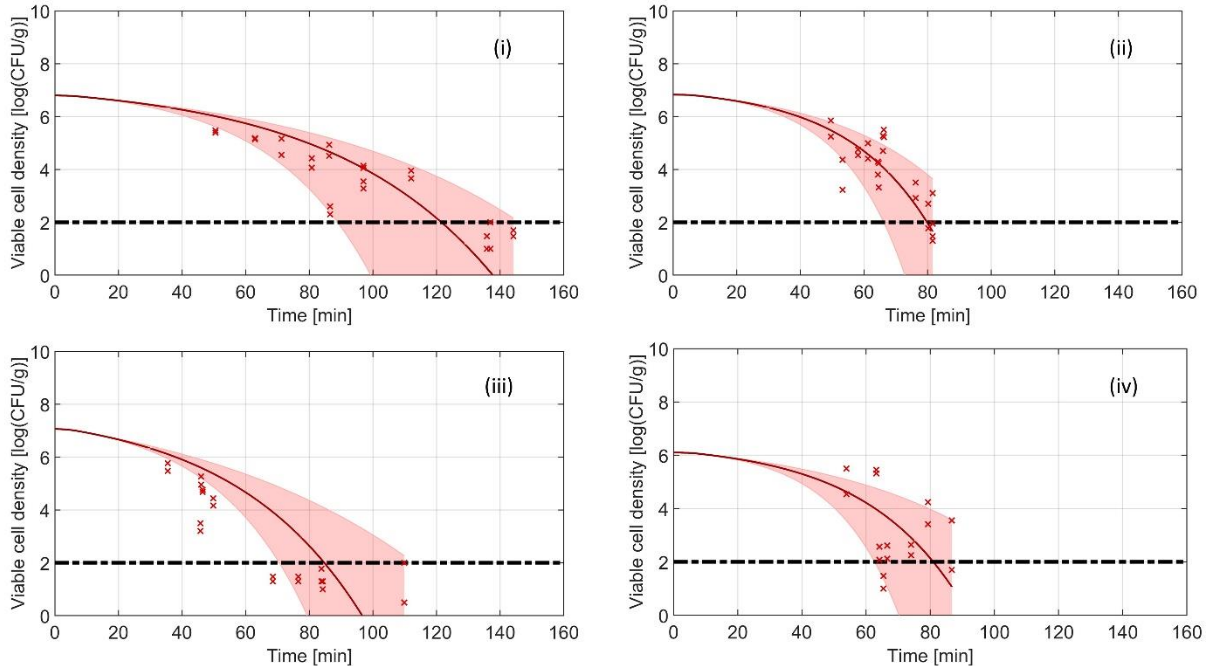
(p<0.05) between RF treated samples in one type of milk powder and different uppercase letters represent significant differences (p<0.05) between the two types of milk powder (n = 2).

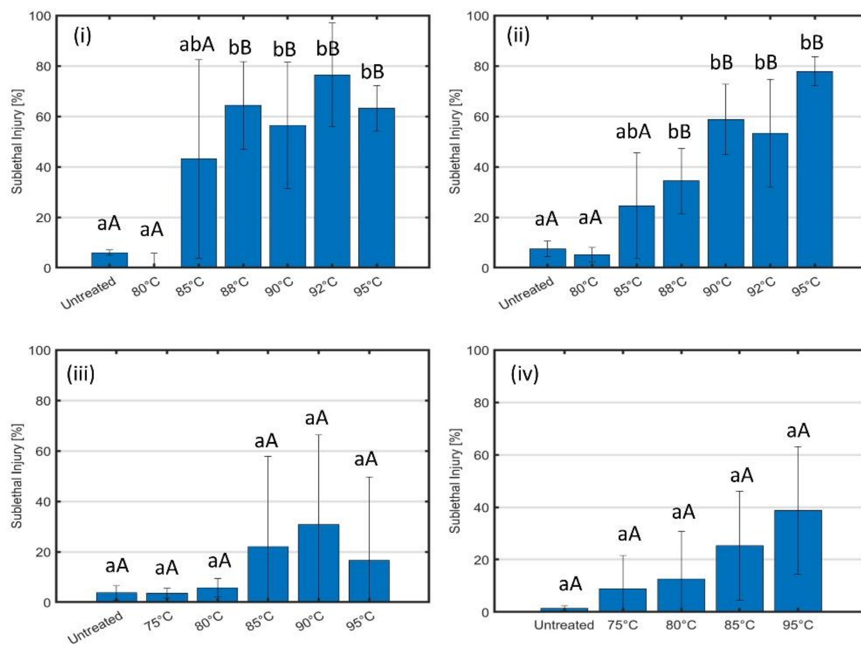
Figure in Supplementary Materials: Cell density (log(CFU/g)) of *E. faecium* in skimmed (i) and whole (ii) milk powder after RF treatment to different end temperatures (n = 2). Data points of black colour represent initial cell density (log(CFU/g)). Data points of different colours represent the cell density (log(CFU/g)) after RF treatment to the respective end temperature. The temperature profile of each treatment corresponds to the specific data points of similar colour. The dashed line represents the detection limit set at 2 log(CFU/g).



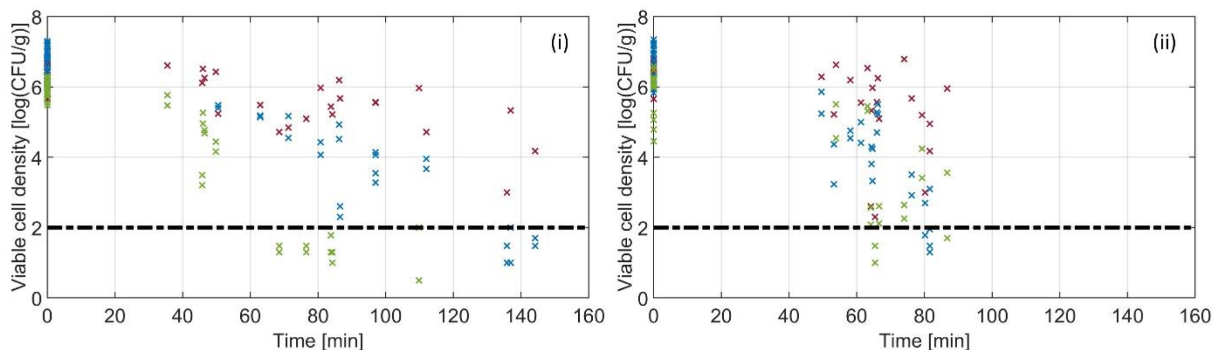

(i)
(ii)
(iii)
(iv)
Viable cell density [log(CFU/g)]
Temperature [°C]
Time [min]

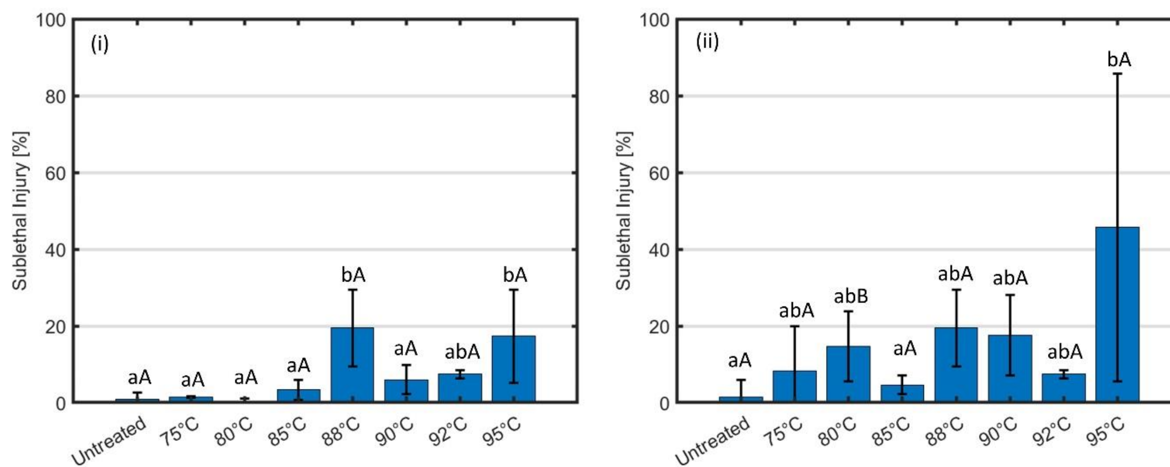

S. Typhimurium in SMP
S. Typhimurium in WMP
L. monocytogenes in SMP
L. monocytogenes in WMP
k [1/min]
T [°C]


(i)
(ii)
(iii)
(iv)
Viable cell density [log(CFU/g)]
Time [min]


(i)
(ii)
(iii)
(iv)
Sublethal Injury [%]
aA
abA
bB
Untreated
75°C
80°C
85°C
88°C
90°C
92°C
95°C

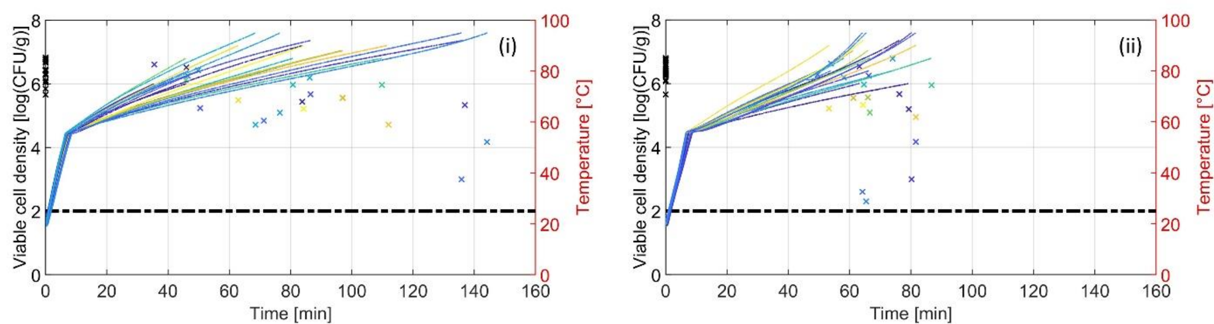
Postprint version of paper published in International Journal of Food Microbiology 2024, vol. 413, nr. 110556.
The content is identical to the published paper, but without the final typesetting by the publisher.





Journal homepage: https://www.sciencedirect.com/journal/international-journal-of-food-microbiology

Original file available at: https://www.sciencedirect.com/science/article/pii/S0168160523004737


Viable cell density [log(CFU/g)]
Temperature [°C]
Time [min]
(i)
(ii)
0
2
4
6
8
20
40
60
80
100
120
140
160